UNITED STATES ENVIRONMENTAL
PROTECTION AGENCY
WASHINGTON, D.C. 20460

September 8, 2017

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

Oxitec, Ltd
c/o Mr. Keith A. Matthews
Wiley Rein LLP
1776 K Street NW
Washington, D.C. 20006

Subject: Comments on the Materials Oxitec Submitted to BPPD on July 7, 2017, Regarding the *Aedes Aegypti* Mosquito Product OX513A

Dear Mr. Matthews:

The Biopesticides and Pollution Prevention Division (BPPD) of the Office of Pesticide Programs (OPP) of the Environmental Protection Agency (EPA) received the materials Oxitec, Ltd., supplied on July 7, 2017. Per my July 5, 2017, discussion with you and Mr. Fred Smith of SciReg, we understand these materials to comprise data and a draft administrative pesticide registration application. We understand that your intent is to resubmit the entire application if FDA's Guidance for Industry (GFI) 236 becomes final and jurisdiction over your product transfers from FDA to the EPA under the Pesticide Registration Improvement Act (PRIA).

For both Experimental Use Permit (EUP) and pesticide registration applications under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), EPA is required to perform an in-depth risk assessment of the application and associated materials (i.e., information and data) to determine whether the application package meets the FIFRA standard of no unreasonable adverse effects, which includes consideration of possible effects on endangered and threatened species under the Endangered Species Act. Upon thorough review of an application, OPP may determine that more information is necessary to complete a risk assessment.

BPPD examined the materials you submitted. We performed a screen, but have not done a full review of the data. We would like to share the results of our preliminary screening of the adequacy of the data to potentially support an application for a FIFRA pesticide registration. As discussed below, BPPD believes the Biochemical Data Requirements are the most appropriate for your product.

Biochemical Data Requirements

Title 40 Code of Federal Regulations Part 158 contains data requirement regulations describing the types of data generally required for a pesticide registration or a pesticide Experimental Use Permit (EUP) for field testing. For the genetically engineered (GE) mosquito, OPP determined that the most appropriate data are those found in Subpart U for biochemical pesticides (see Table 1 below). The active and inert ingredients in Oxitec's GE mosquito are proteins and the biochemical pesticides data requirements are the "best-fit" for evaluating the safety of such proteins. Because OX513A is intended to suppress populations of a public health pest – the *Aedes aegypti* mosquito – efficacy data are also required. Oxitec has generated data that directly address three of the 12 studies in Subpart U. For the other studies, information and laboratory data on the OX513A proteins combined with scientific rationale present a viable approach for attempting to satisfy the remaining non-target and mammalian toxicity data requirements in lieu of conducting the specific studies.

Table 1 - 40 CFR 158 Subpart U "Biochemicals" Data Requirements (158.2010 et seq) + Efficacy

40 CFR 158 Data Requirement	**What Data Did Oxitec Submit?**
Acute oral toxicity - rat	No data to directly address.
Acute inhalation toxicity - rat	No data to directly address.
Primary eye irritation -rabbit	No data to directly address.
Primary dermal irritation	No data to directly address.
Avian oral toxicity	No data to directly address.
Avian dietary toxicity	No data to directly address.
Fish acute toxicity, freshwater	Guppy (*P. reticulata*) study (Fish)
Aquatic invertebrate acute toxicity, freshwater	Study with *Tx. splendens* and *Tx.amboinensis* (Predatory Mosquito)
Terrestrial plant toxicity, seedling emergence	No data to directly address.
Terrestrial plant toxicity, vegetative vigor	No data to directly address.
Non-target insect testing	Some data - *Tx splendens* and *Tx. amboinensis* study (Predatory Mosquito)
Efficacy	Foreign data, but no U.S. data submitted.

Protein Data

The substances engineered into OX513A mosquito consist of proteins (tTAV and DsRed) and the genetic material necessary for the production of these proteins. Protein data address several risk considerations. Specifically, these data help characterize how much GE protein is present in the mosquito, whether the GE proteins are more resistant to breakdown than other proteins, and what physical features characterize the GE proteins, e.g., size, relation to toxic substances and allergens. OPP would use this information to determine how much GE protein the mosquito might introduce into the environment, where the proteins might go in the environment and to answer questions such as what might happen to animals that ingest the GE mosquitoes.

Protein expression data are needed to estimate environmental exposure and provide an estimate of how much of the pesticide is present in the GE mosquitoes and is being put into the environment. Table 2

provides a summary of the protein data that would suffice in lieu of actual mammalian and non-target organism testing. We understand that Oxitec intends to submit this information to EPA in November 2017.

Table 2 – Protein Data and Rationales

OX513A Mosquito Protein	**Protein Laboratory Data or Information Requested**	**Purpose**	**What Data or Information did Oxitec Submit?**
tTAV	Protein expression to support human health and environmental risk assessment	Range of amounts released to environment	Data on protein present in mosquito saliva submitted. Estimate of upper bound of protein expression in mosquitoes was submitted, but actual data from mosquitoes not submitted
tTAV	*In vitro* digestibility in man (pepsin) to support human health risk assessment	How quickly GI tract can break down tTAV protein – the more rapid the less likely an allergenic effect	No data to directly address.
DsRed	*In vitro* digestibility in man (pepsin) to support human health risk assessment	How quickly GI tract can break down tTAV protein – the more rapid the less likely an allergenic effect	Publically available.1
tTAV DsRed	Computer modeling/ bioinformatics analysis to support human health risk assessment	Searching protein sequence databases for similarity to known allergens or toxins	Report of analysis submitted.
tTAV DsRed	*In vitro* testing for breakdown by environmental protease (subtilisin) to support environmental risk assessment	How quickly tTAV and DsRed are broken down in the environment – more rapidly degraded = lower exposure	No data or information to directly address.
tTAV DsRed	Information on protein characteristics e.g., size, charge to support environmental risk assessment	Is tTAV or Dsred likely to pass cell membrane and reach site of action in cell – large, charged molecules unlikely to pass membrane, rapidly digested protein unlikely to pass membrane	No data or information to directly address.
tTAV DsRed	Computer modeling/ bioinformatics analysis to support human health and environmental risk assessment	Searching protein sequence databases for hydrolysis sites for human GI tract and environmental proteases	No analysis submitted.

The information/data you submitted thus far do not appear on their own to be sufficient to support the granting of a pesticide registration for your product under FIFRA section 3. A complete registration application submitted under PRIA will at a minimum need to have the protein data described in Table 2, address U.S. efficacy data listed in Table 1, and additional information, all described below, to be considered complete. In addressing U.S. efficacy data, if you propose that EPA rely on the foreign efficacy data you submitted in lieu of U.S.-generated data, you should include a rationale that compares the conditions of the foreign field data to that which may be found in proposed use areas of the U.S. with

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1 In 2006 Pioneer Hi-Bred International submitted to the Food and Drug Administration (FDA) an Early Food Safety Evaluation on the DsRed protein. The DsRed publicly available FDA submission contains some information OPP judges necessary to support a FIFRA application such as amino acid sequence homology of the dsRed2 protein to known protein allergens, lability of the dsRed2 protein to pepsin in simulated gastric fluid (SGF), and assessment of toxicity potential of the dsRed2 protein (https://www.fda.gov/downloads/food/biotechnology/submission/ucm219002.pdf). OPP is not asking Oxitec to generate data/information found in that submission.

regard to climate, landscape, mosquito density over time, and cultural conditions such as the use of screens and air conditioning.

As you are aware, limited field releases could be done under a pesticide experimental use permit (EUP) and you could formally submit an application for an EPA EUP now before FDA's GFI 236 is final. Most of the information EPA recommended in my June 14, 2017, letter to Tricia Whitmore (see attached) to support an EUP was not included in your July 7, 2017, submission. Should you choose to submit an EUP application, this EUP supporting information, as well as information on indirect effects to non-target organisms, and quality control information regarding arboviruses described below, should be submitted along with the data and information in your July 7, 2017, submission.

For a commercial pesticide application under FIFRA section 3, the application package should also include the data/information discussed below as well.

Human Health and Product Characterization

- To reiterate EPA's concern, registration related protein data, discussed in my letter to Tricia Whitmore dated June 14, 2017 (attached), and summarized in Table 2 above continue to be necessary in BPPD's judgement to support a commercial pesticide risk assessment and were not included in the materials submitted.

- There is no discussion of quality control (QC) for the presence of important *Ae. aegypti*-vectored viruses in the OX513A colony or any QC for the presence of arboviruses in the [redacted] assessment (EA) performed by the Food and Drug Administration, and a rationale for not including QC testing for arboviruses in that submission based on *Aedes* not having spread to the UK. Given the continuing spread of mosquitoes carrying arboviruses into new ranges, such isolation arguments are not tenable. Oxitec should address these QC points.

- Oxitec indicates that penetrance of the tTAV trait is not 100% and that some number less than 5% of the released male mosquitoes can live longer than anticipated in the absence of tetracycline. The information Oxitec means to convey through the term "penetrance" should be explained in greater detail, including phenotype and any potential impact these low penetrance/non-lethality males might have on the *Aedes aegypti* population. Oxitec should address what could happen to the population if released low penetrance/non-lethality males are able to successfully produce viable offspring (i.e., that are able to mature to adults) with wild females. Oxitec should address whether the tTAV trait could in this instance pass stably into the population including to wild females, and what might be the potential consequences of biting females containing the tTAV trait. The implication of this survival rate for both males and any incidentally released females should be addressed since the longevity of the mosquitoes can impact the exposure assessment portion of the risk assessment, e.g., how much does a longer survival affect the potential for non-target organism exposure.

- With regard to the certified limits statement, BPPD requests the information be presented as amount of protein per mosquito.

Ecological Fate and Effects

- In order to perform a robust ecological risk assessment for a biochemical pesticide EPA typically requires and evaluates the studies identified in Subpart U. Given the particular aspects presented by the GE mosquito in regard to exposure to non-target organisms, EPA has previously discussed, in a letter to Tricia Whitmore dated June 14, 2017 (attached), data on the nature and expression of the novel proteins present in the mosquitoes as a way to provide some of the data needed to conduct an adequate risk assessment. These protein data should provide key information regarding exposure and potential toxicity and would obviate the need for several of the typically required studies perhaps saving Oxitec time and expense. Oxitec could alternatively conduct or otherwise address all of the studies outlined in Subpart U, listed in Table 1 above. Although some protein information was provided, additional protein data and information discussed in my June 14, 2017, letter and described in Table 2 above were not included in the material submitted. Neither were studies submitted to fulfill all of the ecological data requirements in Subpart U.

- The submission does not adequately address indirect environmental effects which are a necessary component of an Agency risk assessment of the potential impacts of pesticide use to non-target organisms, including endangered and threatened species under the ESA. OPP has found that not addressing these effects carefully lays the foundation for potential litigation. At our meeting on March 7, 2017, OPP provided a written document (attached) to Oxitec addressing this concern and offering two lines of evidence that Oxitec should discuss in a rationale to address these indirect effects. For example, information on how release of *Ae. aegypti* males affects the numbers of other disease vectors at the release site could help address this point. Information on integrated vector management (IVM) measures that may be used in conjunction with OX513A males could also provide critical information that would enable the Agency to properly assess indirect effects of OX513A males on other disease vectors, and should be part of the submission. This information is especially pertinent to the endangered species assessment.

Efficacy

- OPP typically requires U.S. efficacy data for mosquito control to be conducted in the United States to ensure that the data are relevant to conditions under which the product would be used were it to be registered, before granting a FIFRA section 3 registration. In addressing U.S. efficacy data, if you propose that EPA rely on the foreign efficacy data you submitted in lieu of U.S.-generated data, you should include a rationale that compares the conditions of the foreign field data to that which may be found in proposed use areas of the U.S. with regard to climate, landscape, mosquito density over time, and cultural conditions such as the use of screens and air conditioning.

- For studies conducted in the U.S., Oxitec will need to demonstrate that OX513A males released (eggs originally obtained from Mexico) are not inferior to the wild type males encountered. Fitness tests should be conducted for specific parameters such as longevity, flight duration, competition for females, and anything else Oxitec deems relevant to demonstrating 'lack of

fitness cost'. For studies conducted outside the U.S., Oxitec should provide any data collected that demonstrates a lack of fitness cost. This may help support a weight of the evidence analysis of efficacy.

- Based on the reported sorting accuracy [redacted] BPPD would like to know if Oxitec conducted a sorting trial with positive (female) treatments to test how many females were not captured and passed the sorting process.

- BPPD requests that monitoring information for female adults to measure efficacy of the OX513A male release program be provided to the Agency. OPP mosquito experts have concluded that the endpoint should be number and percent female adults captured after and throughout the release period. BPPD acknowledges that Oxitec considers egg ovitrap data a more accurate measure, however, female adults is the lifestage of concern for the Agency. This information will need to be provided, especially for studies conducted in the U.S. Ovitrap results should accompany adult female results to establish accuracy of that particular tool.

- Please clarify in a future submission if IVM did or did not occur in the background during the conduct of the submitted studies. This is not always clear in the submitted studies (see last bullet point below).

- BPPD further requests that the number of replicates (e.g., ovitraps/release area, BG traps/release area) is communicated in any submission to the Agency.

- For all efficacy studies submitted, please clarify how many ovitraps were placed inside homes and outside homes. Average larvae/ovitrap should be reported separately for the two locations unless Oxitec reports that no barriers existed (e.g., screens) between inside and outside locations.

- For Study 15, 16, 24, 25, 26, and 27: How many BG traps total were used per study area? What is the number of females caught/trap and % female reduction? Please clarify if IVM was present or absent.

Data Formatting

We encourage Oxitec to resubmit your draft studies in the proper data format as soon as possible to help make our review of the data more useful for an actual application. Proper formatting addresses data integrity and compliance with Good Laboratory Practices and makes it possible for EPA to complete its review of that data. Please refer to formatting guidance in PR Notice 2011-3, https://www.epa.gov/pesticide-registration/prn-2011-3-standard-format-data-submitted-under-fifra-and-certain-provisions. Four of the studies submitted to BPPD were compliant with PR Notice 2011-3 and have been assigned permanent MRID numbers. The other studies submitted to BPPD on July 7, 2017, were not formatted correctly and would have to be resubmitted for EPA to complete its review of the data.

Conclusion

As mentioned above, based on BPPD's experience reviewing similar applications in the past, the information/data you submitted do not appear sufficient to support the granting of a pesticide registration for your product under FIFRA section 3. We understand your desire to get your product in the field as soon as possible and note that the EUP process would be quicker, would require less data, and could be started and finished without jurisdiction over your product transferring from FDA to the EPA.

If you have any additional questions, please feel free to contact Dr. Elizabeth Milewski of my staff. She can be reached at (703) 347-0400 or milewski.elizabeth@epa.gov.

Sincerely,

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Enclosures

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